ENZYMATIC PRODUCTION OF CASSAVA FLOUR USING PECTINASE

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ABSTRACT

This paper presents the studies of enzymatic activity of pectinase enzyme in liberation of starch from cassava (*Manihot esculenta*), especially in cassava flour. The starch granules in cassava is hold up by pectin molecules, in which its cell wall can be broken by pectinase enzyme activity. Hence, the assumption is made where the amount of starch released is inversely proportional to amount of pectin molecules left after the enzyme activity. The experiment was designed to determine the optimum condition for the pectinase activity, where parameter of incubation time, temperature, pH of the solution and concentration of enzyme is taken into consideration. The final product, which is the dried cassava powder (flour) is analyzed for equivalent pectin weight by titration method. The flour produced based on optimum conditions was then analyzed for its nutritional content. Analysis of total protein content, moisture content, ash content, crude fat content, crude fiber content were carried out. Based on the experiment, it is found that the optimum condition for pectinase activity is at between 45-50°C temperature, 7 hours of incubation time, pH value of 5 and concentration of 20 mg/ml. At these conditions, the increase in starch release is about 6%, where this value could be higher when more detailed starch assay is carried out. The nutritional profile is also satisfactory, in some cases, very healthy profile, which means the cassava flour fermented with pectinase enzyme have high potential to replace wheat flour.

Key words: Cassava, starch, pectinase, nutritional profile, enzyme, optimum

ABSTRAK

Kertas kerja ini membentangkan kajian aktiviti enzim pectinase enzim dalam pembebasan kanji daripada ubi kayu (*Manihot esculenta*) terutamanya di dalam tepung ubi kayu. Granul kanji dalam ubi kayu adalah memegang oleh molekul pektin, di mana dinding sel boleh dipecahkan dengan aktiviti enzim pectinase. Oleh itu, andaian dibuat di mana jumlah kanji yang dikeluarkan adalah berkadar songsang dengan jumlah molekul pektin tinggal selepas aktiviti enzim. Eksperimen telah direka untuk menentukan keadaan optimum untuk aktiviti pectinase, di mana parameter masa pengeraman, suhu, pH dan kepekatan enzim diambil kira. Produk akhir, yang merupakan serbuk ubi kayu yang kering (tepung) dianalisis untuk berat pektin bersamaan dengan kaedah pentitratan. Tepung yang dihasilkan berdasarkan keadaan optimum dianalisis untuk kandungan nutrisinya. Analisis daripada kandungan protein, kandungan lembapan, kandungan abu, kandungan lemak mentah, kandungan serat mentah telah dijalankan. Berdasarkan eksperimen, didapati bahawa keadaan optimum untuk aktiviti pectinase adalah di antara 45-50°C, 7 jam masa pengeraman, nilai pH 5 dan kepekatan 20 mg/ml. Pada keadaan ini, peningkatan dalam siaran kanji adalah kira-kira 6%, di mana nilai ini mungkin lebih tinggi apabila kanji asai lebih terperinci dijalankan. Profil pemakanan juga memuaskan, dalam beberapa kes, profil yang sangat sihat, yang bermaksud tepung ubi kayu diperam dengan pectinase enzim mempunyai potensi yang tinggi untuk menggantikan tepung gandum.

Kata Kunci: Ubi kayu, kanji, pectinase, kandungan nutrisi, enzim, optimum

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LIST OF ABBREVIATIONS

A.Niger	Aspergillus Niger
AOAC	Association of Analytical Communities
BSA	Bovine Serum Albumin
CAS	Chemical Abstracts Service
FIIRO	Federal Institute of Industrial Research Oshodi
FOA	The Food and Agriculture Organization of the United Nations
HCL	Hydrochloric Acid
HCN	Hydrogen Cyanide
IITA	International Institute of Tropical Agriculture
KOH	Potassium Hydroxide
MSG	Monosodium Glutamate
NaOH	Sodium Hydroxide
ppm	Parts per million
RPM	Revolutions per minute
TPPIA	Thai Pulp and Paper Industries Association
TTSA	Thai Tapioca Starch Association
UV-VIS	Ultraviolet-Visible

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
µm	micrometre
Ca	Calcium
Cal/tan/ha	Calories per tonne per Hectare
g	Grams
K	Potassium
mg/ml	Milligrams per Millilitres
mm	millimetre
Mt/ha	Metric Tonnes per Hectare
nm	nanometre
pH	Power of Hydrogen
Sp.	Species
Tons/ha	Tonnes per Hectare
w/w	Weight by Weight

1 INTRODUCTION

1.1 Background

Increasing population of human being have also increased the demand for staple food with rice, maize and wheat providing two third of them currently. Cassava, meanwhile, have the ability to become the substitute for all of them based on its low production cost, drought-tolerance, high-productivity per unit land, and high nutrients content. The low-in-protein but carbohydrate rich crop, represent an important energy source and staple food source for more than 500 million people throughout tropical Africa, Latin America and certain parts of Asia (Hock *et al.,* 1998). Being the large carbohydrate source, cassava is produced largely in Brazil industrial purpose and as export crop in Thailand. In Africa however, cassava is produced primarily for food consumption making it the most important crop in the continent (Fauquet *et al.*, 1990).

Cassava has emerged has food security for most of the developing country due to its ability to tolerate adverse environmental conditions to grow in diverse ecosystem. It grows best in high rain-fall area (annual rain-fall of 1000-2000 mm) but tolerates drought in low rain-fall area mainly in Africa. Soil type is not a concern for cassava growth as it can grow in soil regardless of pH ranging from alkaline to acidic and marginal or high-productive soil.

The principal parts of cassava plant are roots (50%), stem (44%) and leaves (6%) with the roots and leaves being nutritionally valuable parts which have potential as a feed source. The major carbohydrate source, the roots, consists of 60-65% moisture, 20-31% carbohydrates, 1-2% crude proteins, 0.2-0.6% ether extracts and very low content of minerals and vitamins. However, calcium and vitamin C is rich in cassava roots. Though the quantity is low, the protein quality is high given that the proportion of essential amino acid of total nitrogen is high (Olumide, 2004).

Although the processing of cassava roots yields stable products with the removal of most of the cyanogens, there is still retention of 12-33% of cyanide in cassava flour produced in eastern, southern and central Africa, whereas a relatively low magnitude (1.8-2.4%) of cyanide found in flour produced in West Africa and southern America (Cardoso *et al.*, 2005). Konzo, an epidemic paralytic disease, associated with consumption of cassava flour of high cyanide content (Ministry of Health Mozambique, 1984). Konzo recorded high in Mozambique due to the low-rainfall in the country, as the cyanide content found to be doubled than to an average content (Cumbana *et al.*, 2007). The degree of gelatinization in cassava starch affects its physical properties such as bulk density and volume, and these changes in the properties can affect the primary use of cassava starch as flour.

1.2 Motivation& Statement of the problems

Pectinase is common enzyme used in food industries. It has been widely used in juice clarification as juice mainly contains polysaccharides such as pectins, cellulose and starch (Wong *et al.*, 2009). During the enzymatic treatment, pectinase breaks down the pectin molecules that led to a reduction of water holding capacity and consequently, free water is released to the system and reduces the viscosity (Lee *et al.*, 2006). Using of pectinase in starch extraction said to give better release of starch without giving any significant changes to the physical properties of the starch. Espino *et al*. (2005) studied that the use of cellulose and pectinase in the extraction of starch from cassava and sweet potato increased the percent yield and subsequent recoveries without compromising the quality of the starch produced.

However, cassava has some minus points as well. Raw cassava flour, which is actually the powder form of cassava roots, have only about 25% of starch readily available (TTSA., n.d). Relatively lower amount of starch reduces the economic value of cassava flour and at the same time affects the primary use of flour as the gelatinization factor is lesser. Hence, the focus is now on releasing maximum amount of starch from cassava. Enzymatic starch liberation using pectinase is chosen as the pectinase's primary function is to break the pectin molecules which hold up starch granules (Lee *et al.*, 2006). The objectives of the studies is to find out the optimum condition for enzyme activity in breaking the pectin molecules.

Considering climate in Malaysia, the high average annual rain-fall ranging from 1781 mm - 4159 mm (Malaysian Meteorological Department., 2010) would provide the best condition for growth and for production of starch with minimal cyanide content. Pectinase meanwhile can be used to reduce the gelatinization in the starch. Bearing these two factors, Malaysia can optimize the climate condition to become ideal producer of cassava starch worldwide.

1.3 Objectives

The work aims to produce flour from cassava by using pectinase enzyme that meets standards of flour and compare it to that of wheat flour.

1.4 Scope of this research

The study will be focusing on conducting test for each properties mentioned. The sample (roots/tubers) will be collected manually using traditional way of peeling, washing, drying, grinding and adding pectinase. The similar sample will be used for all tests with slight modification (if needed). The area of study narrows to the following:

a) To study the enzymatic starch liberation from cassava by pectinase, whereby the study will focus on identifying the optimum condition for the pectinase activity.
b) To test for nutritional profile of starch (moisture, protein, fat, fiber and ash) where the tests will be carried out on flour produced based on optimum condition.
c) To compare the test results to the specification of standard wheat flour

1.5 Main contribution of this work

The main contribution of the work will be determining the possible potential of pectinase to be used as enzyme to liberate starch which eventually will help to produce flour that meets standard specifications.

1.6 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 1 provides slight introduction of the cassava plant and cassava flour. The issues related to the cassava flour are also studied where it leads to our motivation and problem statement. This chapter also provides the objectives of the research and the scope of the research to achieve the objective.

Chapter 2 gives a review of previous studies related to the topic. The studies covers applications of cassava, production of cassava plant, the nutritional profile of cassava flour, the degree of gelatinization and the toxicity of cassava flour. This chapter also provides studies about pectinase enzyme.

Chapter 3 is a detailed description of the methodology for the research. It provides explanation on the sample preparation for different parameter studies and the method for analysis.

Chapter 4 provides the overall finding of the study with detailed description for each parameters studied and each analysis made.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

2 LITERATURE REVIEW

2.1 Overview

Studies have been on going to optimize the production of cassava starch with specifications that meets standard requirement and security. Improvement on cassava limitations become our focal point of study with the possible outcome could change the current dependency of the world population towards rice, wheat and maize and staple food.

2.2 Cassava

Cassava, with the scientific name of *Manihot esculenta,* also called as tapioca when it is dried to a starchy and powdery extract. Cassava is also known as '*yuca*' in Philippines, '*tabolchu*' in North East India and as '*mogo*' in Africa. The leaves of can be consumed, but it is the tubers, or actually its swollen root, that being harvested the most for consumption. The adult cassava plant can grow up to 3 metres tall. The cassava plant is mostly propagated through stem cutting. (Tonukari., 2004)

Figure 2-1: A cassava (*Manihot esculenta*) tuber or root, the main part of the plant.

2.2.1 Production of cassava

Global production of cassava as of 2005 is at the rate of about 160 million tons per year Nigeria is the world largest cassava producer; however Thailand is the number one exporter of dried cassava, where Thailand owns 77% of the total world cassava export in 2005. (FOA., 2008) Thailand is followed far behind by Vietnam, India and Costa Rica in the aspect of exporting country. In Nigeria, almost all the cassava produced is used for human consumption and less than 5% is used in industry. (Ukwuru & Egbonu., 2013)

High number of production in Africa is encouraged by the ability of the cassava plant to tolerate drought, a special character that is rare among all other crops. This means that the cassava plant can be grown in the areas of least annual rainfall and also in the place where the soil quality is said poor with pH ranging 4-9. (Okigbo., 1980)

Okigbo (1980) also identified few other reasons for the rapid growth of cassava plant in Africa besides drought tolerating ability and adaptation to poor soil. Cassava is easily propagated through stem cutting, unlike other major crops. Another major plus point of cassava plant is that is relatively high yielding plant and excellent source of calories. According to DeVries (1967), the potential yield of cassava can be up to 250,000 calories/ha/day and 75 tons/ha.

Figure 2-2: A cassava farm in Nigeria, Africa

2.2.2 Future of Cassava Production

Sub-Saharan Africa is expected to experience the most rapid growth in food demand in root and tubers averaging 2.6 percent per year through 2020 (Scott *et al*., 2000). This growth will account for nearly 122 million metric tons with most of the increase coming largely from cassava, 80 million metric tons (66% of the total). Cassava demand is estimated to grow at 2.0% annually for food and 1.6% per year for feed in developing countries, while total cassava production is projected to reach 168 million tons by 2020 based on the current production rate. However, according to Tonukari (2004) this amount can be far surpassed in developing countries with the right policies and incentives. Current production rate (1993) and estimated production rate in 2020 are shown in table 2.2.

Table 2-1: Cassava Production and Use in 1993, and Projected to 2020

Country/region	Area (million ha)		Yield (mt/ha)		Production (million mt)		Total use (million mt)	
	1993	2020	1993	2020	1993	2020	1993	2020
Sub-Saharan Africa	11.9	15.9	7.4	10.6	87.8	168.6	87.7	168.1
Latin America	2.7	2.7	11.3	15.6	30.3	41.7	30.3	42.9
Southeast Asia	3.5	3.5	12.1	13.7	42.0	48.2	18.9	24.4
India	0.2	0.2	23.6	28.4	5.8	7.0	5.7	7.3
Other South Asia	0.1	0.1	9.4	13.5	0.8	1.3	0.9	1.4
China	0.3	0.3	15.1	20.2	4.8	6.5	5.1	6.4
Other East Asia	na	na	na	na	na	na	1.8	1.9
Developing	18.8	22.9	9.2	12.0	172.4	274.7	152.0	254.6
Developed	...	...	12.1	14.7	0.4	0.4	20.7	20.5
World	18.8	22.9	9.2	12.0	172.7	275.1	172.7	275.1

2.2.3 Cassava as Food Source

Cassava crop provides an important food security to the people in Africa especially for those living in the belt of cassava plant farms. Given the main advantage is drought-tolerance, countries which has low annual rainfall, such as Nigeria and Congo, depend solely on cassava as their main energy source. The term 'security' is perfect for cassava plant as the crop or main part of the crop, grows conveniently underground. This means the invaders cannot easily destroy or remove the crop. (IITA., 2009)

Figure 2-3: Cassava is crucial to the food security of millions of people in sub-Saharan Africa

Nearly every person in Africa is consuming about 80 kilograms of cassava per year and it is estimated that 37% of dietary energy comes from cassava. According to International Institute of Tropical Agriculture (IITA), The Democratic Republic of Congo is the largest consumers of cassava in sub-Saharan Africa, followed by Nigeria.

Supplying 38.6% of caloric requirement in Africa in 1970's, which the figure is on the rise to date, cassava crop are now being consumed all over the world where the number of people relying on it reaches 500 million already. (Philips., 1974) In Nigeria especially, cassava roots are used for the preparation of *Gari* (fermented version of tapioca), *fufu* (dough and porridge-like food prepared from cassava), tapioca cakes (starch extracted from cassava) and of course the cassava flour, which possesses normal application of flours. (Etejero & Bhat., 1985)

Figure 2-4: Fufu: A Traditional Cassava Food in Nigeria

Figure 2-5: Gari: Another Traditional Cassava Food in Nigeria

2.2.3.1 Cassava Bread

The use of cassava flour as a raw material for the bakery and pastry industries is fast growing and gaining recognition as reliable partial substitute for wheat. Using high quality cassava flour particularly is suitable since it has no fat content which is important for storage life. Other possible advantages include its bland taste offering no foreign odour or flavour.

Figure 2-6: Cassava Bread or 'Bread of Tropics' in African Countries

Khalil *et al*. (2000), specifically reported that inclusion of cassava flour into wheat flour up to about 30% could still give an acceptable fresh loaf depending on the source of the flour. Federal Institute of Industrial Research Oshodi (FIIRO) Nigeria, has developed cassava bread with 20% high quality cassava flour substituted with 80% wheat flour, which gives similar characteristics of bread produced with 100% wheat flour both in sensory and nutritional properties. The use of composite flour will enable the developing countries, especially in countries where cassava is readily available, to save some scarce foreign exchange expanded on importing flour (Ogunsua., 1989).

2.2.3.2 Cassava Cookies

The need for strategic development and use of inexpensive local resources in the production of popular foods such as cookies has been recognized by organizations such as the Food and Agricultural Organization (FAO), the International Institute for Tropical Agriculture (IITA), Nigeria and the Federal Institute for Industrial Research Oshodi (FIIRO), Nigeria (Falola *et al.*, 2011). Research at IITA has shown that cassava flour (100%) can be used to prepare bakery products such as cookies and doughnuts (Onabolu and Bokanga., 1998). The resulting products are readily available and sold in Nigeria, thus helping to improve food and livelihood security.


Figure 2-7: Cassava Cookies in Brazil

2.2.3.3 Cassava Biscuits

Cassava flour proved effective as a partial substitute for imported wheat flour in biscuits. High quality cassava flour can substitute for up to 30% of wheat flour in sweet dough biscuit and 40% in hard dough biscuit, without consumers being able to detect any adverse change in colour, taste or texture when compared to 100% wheat flour control (Oyewole., 2002). Substitution of more than 40% wheat flour by cassava in biscuits affects the texture and crispiness. Researches are ongoing to find a good solution to finally make cassava a complete wheat substitute in biscuits.

Figure 2-8: Biscuits made from Mixture of Tapioca, Buckwheat and Rice Flour

2.2.4 Secondary Usage of Cassava

Apart from being major consumable energy source, cassava plant also offers various benefits and application to the world. The usages of cassava have been expanded to the industries such as starch, textile and fuel. The usage of cassava have been summarised in Table 2-1

Table 2-2: Cassava Products and Major Uses

Major uses	**Products**
Human consumption	Raw cassava
	Boiled cassava
	Cooked cassava slices
	Fried cassava slices
	Cassava flakes
	Fermented cassava
	Cassava flour

	Macaroni
	Fufu
	Gaplek
	Composite flour, bread
	Tapioca
	Gari
	Cassaripo or tucupa
	Cassava rice
Livestock feed	Cassava pellets
	Cassava meal
	Cassava chips
	Cassava slices (fresh or boiled)
	Cassava peels
	Cassava-leaf meal
	Broken roots
	Cassava silage
Industrial products	Starch
	Alcohol
	Glucose
	Acetone
	Dextrins
	Glues and pastes
	Binders
	Stabilizer
	Bodying agent (caramel)
	Fillers
	Dusting agent (chewing gum)
	Single-cell protein

Source: Archives of The United Nations University website.

Figure 2-9: Cassava chips for livestock feed

2.2.5 Cassava in Biofuels Production

Cassava is a good feed stock to produce ethanol because it has high starch content. Cassava starch can be converted readily to ethyl alcohol in a two-stage process involving the hydrolysis of starch slurry into glucose by liquefaction so that dextrin and subsequently fermentable sugar can be obtained. The glucose solution is diluted and converted to ethyl alcohol by the anaerobic action of yeast, ethanol of 95.6% w/w comes out through dehydration which is concentrated to 99.5% w/w (Ramasamy and Paramasamy., 2001). Thus cassava-based fuel ethanol is produced and it is usually denatured by small volume of gasoline or other materials added preventing people from drinking it, (Leng *et al*., 2008).

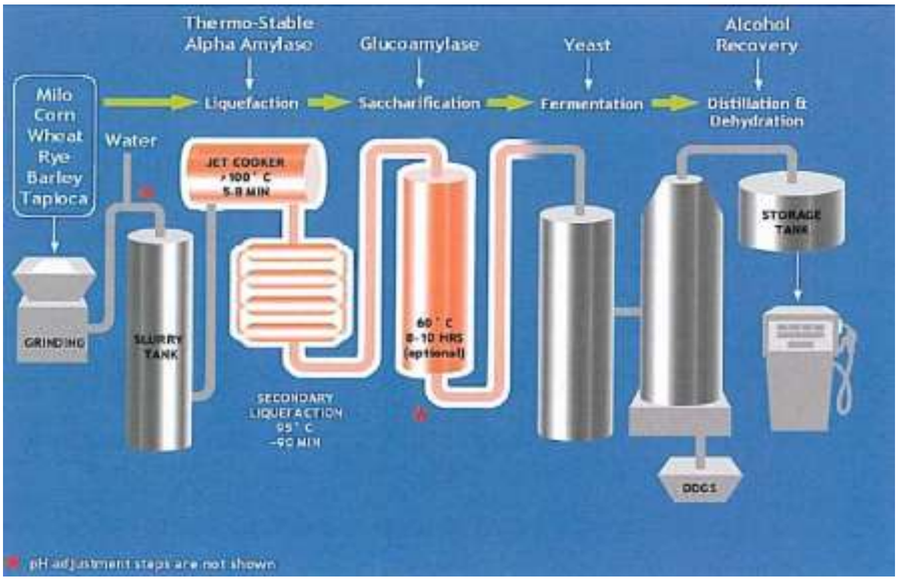

Figure 2-10: Conventional Ethanol Production Process

The ethanol produced is of high quality similar to cereal alcohol. Saccharification can be accomplished either by hydrolytic process or the biological process. The hydrolytic process uses hydrochloric acid or sulphuric acid. Yields are low and continuous use of acid causes equipment corrosion and is dangerous to handlers. The biological process uses amylolytic enzymes, which can be obtained from barley malt and moulds that grow on rice or wheat bran. This process results in higher yields than the acid process.

Figure 2-11: How to make biofuel in five easy steps (left-to-right): chipped cassava stalk; milled cassava stalk; after pre-treatment; after enzyme hydrolysis; post-fermentation – the bioethanol "beer".

Table 2-3: Some advantages of Biofuel Produced from Cassava Starch

It is not poisonous
It does not cause air pollution or any environmental hazard
It does not contribute to the green house effect problem (CO_2 addition to the atmosphere, causing global warming)
It has a higher octane rating than petrol as fuel. That is, ethanol is an octane booster and anti-knocking agent
It is an excellent raw material for synthetic chemicals.
Ethanol provides jobs and economic development to rural areas.
Ethanol reduces country's dependence on petroleum and it is a source of non – oil revenue for any producing country
Ethanol is capable of reducing the adverse foreign balance trade.

2.2.6 Processing of cassava

Cassava plant, before being processed for multi-purposes, especially the tuber part, will need to undergo several simple steps. Processing begins with harvesting of the roots where the woody end of the roots (stem) is cut off. The roots must be processed in 2 days in order to get optimum products.

Figure 2-12: Cassava is carried to Tinh Phong Cassava Starch Processing Factory in Quang Ngai Province, Vietnam

The process is then continued by peeling the skin of the roots and washing. Removal of water from the cassava is done by drying for certain hours. Then the cassava tubers are cut into small pieces before being grinded into powdered form. In the case where fermented starch is preferred (for most of the cases fermented starch is preferred, selected enzyme will be added in the powdered form of the cassava and fermented. The fermentation process is essential here in order to reduce cyanogenic glycosides, which will release hydrogen cyanide, HCN content of the flour produces. According to Tewe (n.d.), fermented cassava products store better and often are low in residual cyanide content.

Figure 2-13: Grated cassava and cassava flour